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Suppression of Sebaceous Gland Development in Laboratory Animals by 17α-Propyltestosterone

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Topical application of the testosterone derivative 17α-propylandrost-4-en-17β-ol-3-one (Win 17665) caused a dose-related regression of the hamster flank organ and guinea pig supracaudal gland in mature male animals. Histological examination confirmed that this action of Win 17665 was on the size of the hamster sebaceous glands and was reversible on cessation of treatment. Topical application of Win 17665 also counteracted flank organ stimulation by directly applied 5α-dihydrotestosterone and 4-androstene-3,17-dione. Seminal vesicle weight was reduced after the repeated subcutaneous administration of 100 mg/kg of Win 17665 but not after repeated oral administration of 320 mg/kg or topical administration of 1 gm/kg of Win 17665.

Since androgens play a central role in the etiology of acne [2], the development of agents that counteract the response of sebaceous glands to increased levels of androgens during and after puberty would be a rational approach to therapy. However, antagonism of systemic androgen activity would severely limit the general use of any such therapeutic agent in the treatment of acne.

Our search for antiandrogens that would antagonize the enlargement of sebaceous glands on topical administration without manifesting noticeable systemic antiandrogenic activity produced one steroid with the desired properties, 17α-propylandrost-4-en-17β-ol-3-one (Win 17665), a testosterone derivative. The animal models used to elicit the antiandrogenic properties of Win 17665 were the hamster flank organ [3] and, to a lesser extent, the supracaudal gland of the guinea pig [4,5].

MATERIALS AND METHODS

Intact or castrated adult Syrian hamsters were medicated topically with Win 17665 in 5 μl ethanol. Controls were either unmedicated or given ethanol vehicle as indicated. The hair in the region of the flank organ was clipped as necessary to expose the region for medication and evaluation. The following parameters were used to evaluate antiandrogenic activity.

Size

The diameter of the asymmetrical flank organ was measured to within 0.1 mm *in situ* with vernier calipers. Measurements were uniformly taken parallel to the breadth of the hamster.

Development

Overall flank organ development was assessed subjectively according to degree of pigmentation, coarseness of hair stubble, area and elevation graded from 0 to 4 as follows: 0—barely discernible flank organ, 1—minimal, 2—moderate, 3—marked and 4—maximal development. Median flank organ development scores were determined for groups by the non-parametric statistical procedures given below.

Flank Organ Weight and Cholesterol Content

Cholesterol is a major constituent of animal sebum [6] and of cell membranes which increase in size and number during androgen-induced hyperplasia of the sebaceous gland. At the end of each experiment, the flank organs were removed, weighed, digested in alcoholic KOH and extracted twice with hexane. An aliquot of the extract was dried and the cholesterol content was determined colorimetrically [7].

Endocrine Organ Weights

Seminal vesicles (with the coagulating gland and seminal fluid removed), adrenals, thymus, and in some experiments, testes were weighed as an index of systemic hormonal activity after topical or parenteral drug administration.

Histology

One flank organ was fixed in formalin. One-half was stained by the Trichrome method and the mean area of the 10 central (the largest) sebaceous glands was determined in sections as an index of glandular size. The other half was frozen-sectioned and stained with Sudan IV dye for determining lipid content. The diameter along 2 axes was measured with an ocular micrometer (125×) and the area calculated.

All but 2 of the experiments were conducted by a blind experimental design. Drug and vehicle solutions were coded by someone not involved with animal medication or evaluation. After the analyses had been completed, the code was broken and the results were compared statistically. The 2 exceptions are noted in the tables.

Guinea Pig Study

The posterior dorsal area of mature male guinea pigs was shaved and the supracaudal gland was measured. Win 17665 was applied topically according to the same procedure for hamsters. Weights of excised glandular regions, seminal vesicles and adrenals were obtained at the end of the experiment.

Statistical Analyses

Distribution of flank organ development scores were contrasted by the nonparametric Kruskal-Wallis analysis of variance by ranks; the data are presented as the median development score. All other parameters were analyzed assuming normal distributions. If nonhomogeneity of variances was detected within the analysis of variance, a Beheren's-Fisher correction was employed in specific *t*-test contrasts in place of the pooled error term.

RESULTS

Topical Application of Win 17665 to Male Hamsters

Win 17665 was applied topically to 1 of 2 flank organs of mature male hamsters for 3 weeks (Table I). A dose-related decrease in flank organ measurements was obtained on the treated side but no statistically significant changes in the contralateral, vehicle-treated side occurred compared with Group I which received no Win 17665. In another experiment in which Win 17665 was applied to both flank organs between 10 μg and 400 μg, similar results were obtained and seminal vesicle, adrenal and thymus weights were unaffected. Therefore, the effect of Win 17665 was local rather than systemic following topical administration. When topical doses of Win 17665 ranging from

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Abbreviations:
AED: 4-androstene-3,17-dione
DHT: 5α-dihydrotestosterone
TP: testosterone propionate

TABLE I. *Unilateral topical application of Win 17665 on the flank organ of mature male hamsters*[a]

	Group and treatment	Diameter[b] (mm)	Weight[b] (mg)	Cholesterol[b] (μg)	Development score[c]	
					Initial	Final
I	Vehicle	5.76 ± 0.26	47.0 ± 4.2	224 ± 23	3	4
	Vehicle	5.86 ± 0.32	47.7 ± 3.6	222 ± 21	3	4
II	Vehicle	5.70 ± 0.17	42.6 ± 2.0	206 ± 14	3	4
	10 μg Win 17665	4.42 ± 0.12[e]	30.9 ± 1.7[d]	155 ± 9.5[d]	3	2[e]
III	Vehicle	5.41 ± 0.13	45.7 ± 2.2	207 ± 14	3	4
	50 μg Win 17665	4.12 ± 0.06[e]	24.0 ± 1.7[e]	143 ± 12[d]	3	2[e]
IV	Vehicle	5.56 ± 0.21	47.4 ± 4.7	222 ± 18	3	4
	250 μg Win 17665	3.78 ± 0.13[e]	21.0 ± 1.5[e]	153 ± 7.3[d]	3.5	1[e]

[a] Groups of 8 hamsters each were treated topically with 5 μl of ethanol vehicle on 1 flank organ or the indicated amount of Win 17665 dissolved in ethanol, on the other flank organ daily for 3 weeks.
[b] Mean ± S.E.
[c] Median scored between 0 for barely visible to 4 for maximum development.
[d] $P < 0.01$ or [e] $P < 0.001$ comparing treatments within group.

0.5 to 10 μg/day were applied to hamster flank organs, the minimum effective dose which caused a significant inhibition of all flank organ measurements was 10 μg and the maximum ineffective topical dose was 0.5 μg.

Similar experiments, not shown, were performed with castrated male hamsters and mature female hamsters supplemented topically or subcutaneously with testosterone propionate (TP), with varying amounts of Win 17665 applied topically. In all cases, Win 17665 produced a dose-related suppression of the flank organ parameters.

Reversal of established androgenic stimulation of the hamster flank organ as in normal mature males took place at the same dose levels of Win 17665 as those required for prevention of development in immature males or in TP-supplemented castrates.

Regression of Sebaceous Glands and Extent of Reversibility

Hamsters were treated topically with Win 17665 for 3 or 6 weeks. Flank organs were examined microscopically for histological changes in sebaceous gland size at 0, 3 and 6 weeks. Two hundred μg/flank organ of Win 17665 caused a highly significant ($P < 0.001$) decrease in sebaceous gland size both at 3 and at 6 weeks of treatment (Table II). The sebaceous gland cells from the untreated control group were numerous and the hair bulbs were thick and pigmented. The large sebaceous gland mass compressed the dermal melanocytes into small, compact collections. The sebaceous glands filled most of the thickened dermis and the cells contained large amounts of Sudan IV-stainable lipid. In contrast, glands in tissue sections from hamsters treated for 6 weeks with 200 μg/flank organ of Win 17665 regressed to those found in immature hamsters. The amount of lipid that stained with Sudan IV was considerably less than that found for untreated control flank organs. These sebaceous glands were poorly developed and the overlying epidermal layers were thin.

In order to test the extent of reversibility of flank organ regression after topical treatment with Win 17665, hamsters that were medicated blind with either vehicle or with 200 μg Win 17665/flank organ for 6 weeks were observed for an additional 6 weeks after vehicle or drug application was discontinued (Fig 1). During the 6-week treatment period the sebaceous glands regressed; after cessation of treatment, glandular areas reverted to the size observed in the vehicle control group.

Effect of Oral, Subcutaneous or Massive Topical Administration of Win 17665 on Flank and Endocrine Organ Parameters

Win 17665 was administered orally at 5, 20, 80 and 320 mg/kg body weight for 21 days (Table III). Body weight gain and thymus weight were reduced ($P < 0.01$) at the 320 mg/kg dose only while adrenal and seminal vesicle weights were unchanged at all doses tried; however at the highest dose (320 mg/kg/day), all of the flank organ parameters were reduced significantly.

TABLE II. *Mean area of the sum of 10 central sebaceous glands in sexually mature male hamsters*

Weeks on test[a]	Mean area of 10 glands $10^4\mu^2$ ± S.E.		% decrease
	Control	Win 17665	
0	19.58 ± 1.58	—	—
3	24.03 ± 1.36	12.15 ± 1.12[b]	49
6	18.11 ± 1.62	6.07 ± 0.93[b]	67

[a] Eight animals per group. Control hamsters were untreated. Groups treated with Win 17665 received 200 μg on each flank organ for indicated time periods.
[b] $P < 0.001$ vs. respective control.

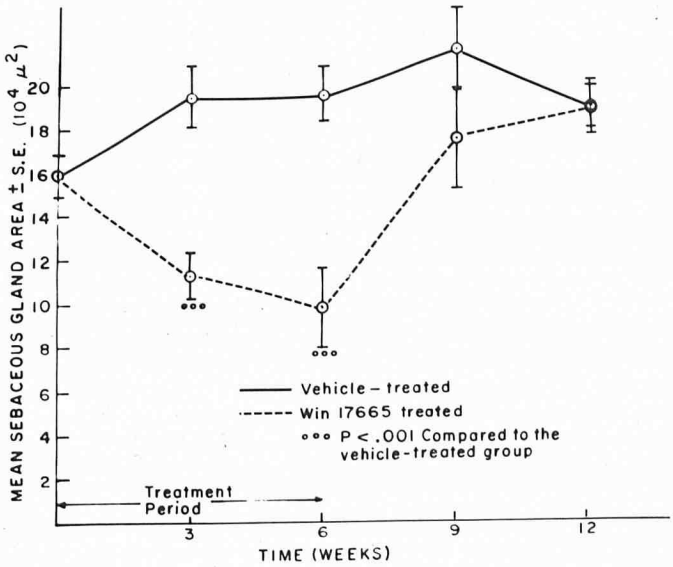

FIG 1. Area of 10 central sebaceous glands during and after topical treatment with 200 μg Win 17665.

The median flank organ development score was also reduced significantly in response to the 20 and 80 mg/kg dose.

In the subcutaneous study (Study 1, Table IV), Win 17665 was injected daily at 1, 10 or 100 mg/kg body weight for a 15-day period. Body and testicular weights were not affected but seminal vesicle weights were decreased ($P < 0.01$) by 28% at the 100 mg/kg dose. Flank organ parameters, except cholesterol content, were also significantly reduced ($P < 0.001$) at 100 mg/kg.

Fifteen days of daily topical application on the shaved backs of hamsters with 100 or 1000 mg/kg body weight of Win 17665 in a volume of 0.2 ml, expectedly and significantly reduced flank organ parameters (Study 2, Table IV); however, seminal vesicle

TABLE III. *Effect of Win 17665 given orally on body weight gain, endocrine organ weights and flank organs of male mature hamsters*

Oral Win 17665 mg/kg/day × 21[a]	Body weight gain (gm) mean ± S.E.	Mean organ weights (mg) ± S.E.			Flank organ parameters ± SE			
		Seminal vesicle	Thymus	Adrenal	Mean diameter (mm)	Mean weight (mg)	Mean cholesterol (μg)	Median development score[b]
Vehicle	12.10	228.5	49.39	22.11	4.86	31.40	220.7	3.75
	±0.95	±8.61	±2.56	±1.11	±0.14	±2.06	±11.6	
5	15.70	224.3	43.38	23.66	5.10	37.65[c]	179.2[d]	3.50
	±1.75	±6.34	±2.19	±0.98	±0.15	±2.37	±11.0	
20	14.30	233.5	47.31	22.69	4.73	37.82[c]	249.0	2.75[c]
	±1.73	±8.52	±1.96	±0.74	±0.16	±2.41	±12.7	
80	10.30	223.4	43.98	23.96	4.74	33.70	194.0	2.75[c]
	±1.89	±7.90	±1.38	±0.87	±0.17	±2.15	±11.6	
320	5.80[e]	230.0	38.96[d]	21.62	4.30[e]	24.68[e]	145.0[e]	2.00[e]
	±1.04	±7.28	±2.20	±0.85	±0.12	±1.29	±7.7	

[a] Each group had 10 animals of mean initial body weight of approximately 100 gm. The hamsters were treated twice on Mon. and Fri., once on Tues. through Thurs., and not on Sat. or Sun.
[b] Scored between 0 for barely visible to a high of 4 for maximum development.
[c] $P < 0.05$; [d] $P < 0.01$; [e] $P < 0.001$ compared with the mean of the control group.

TABLE IV. *Effect of Win 17665, subcutaneous or topical on excised flank organs, body weights, seminal vesicles and testes of mature male hamsters*

Treatment (mg/kg/day[a] × 15)	Mean final body wt (gm) ± SE	Mean organ weights		Flank organ parameters ± SE			
		Sem Ves (mg) ± SE	Testes (gm) ± SE	Mean diameter (mm)	Mean weight (mg)	Mean cholesterol (μg)	Median development score[d]
Study 1—subcutaneous							
Vehicle[b]	124.1	265.7	3.40	4.88	38.1	218.8	4.0
	±2.8	±10.4	±0.16	±0.10	±1.5	±10.6	
Win 17665[1]	127.7	266.7	3.32	5.34	34.5	268.5	3.0
	±3.9	±9.1	±0.06	±0.14	±2.2	±15.3	
Win 17665[10]	126.3	258.9	3.32	4.88	28.5[f]	215.7	4.0
	±2.4	±11.9	±0.11	±0.15	±1.9	±22.6	
Win 17665[100]	121.8	192.1[f]	3.15	4.06[f]	16.5[f]	219.4	2.0[f]
	±2.3	±12.9	±0.08	±0.15	±0.9	±13.0	
Study 2—topical							
Vehicle[c]	113.6	237.0	3.05	5.14	33.4	224.3	4.0
	±2.6	±10.3	±0.07	±0.10	±1.6	±9.4	
Win 17665[100]	114.2	228.6	3.02	4.09[f]	19.6[f]	186.0	2.0[f]
	±2.2	±7.9	±0.08	±0.15	±1.6	±15.0	
Win 17665[1000]	113.1	225.2	3.07	4.50[e]	22.2[f]	186.1	2.5[e]
	±2.6	±7.3	±0.09	±0.13	±1.8	±16.2	

[a] Ten hamsters per group were medicated twice on Mon. and Fri., once on Tues. through Thurs. and not on Sat. or Sun.
[b] Cottonseed oil, 10% ethanol. [c] Ethanol. [d] Scored between 0 for barely visible to 4 for maximum development. Compared with vehicle control: [e] $P < 0.01$; [f] $P < 0.001$.

and testicular weights were unaffected, suggesting that the rate of penetration was slow enough and/or the rate of metabolism was rapid enough to annul the systemic activity of this antiandrogen when the route of administration was topical.

Effect of Win 17665 as an Antagonist of Other Androgens

Win 17665 was tested as an antagonist of 2 naturally occurring androgens, 5α-dihydrotestosterone (DHT) and 4-androstene-3, 17-dione (AED) compared with TP in castrated hamsters. Win 17665, applied topically for 2 weeks at 25, 75 or 225 μg caused a dose-related inhibition of the flank organ parameters stimulated by 1 μg of TP, 1 μg of DHT or by 5 μg of AED (Table V).

Antiandrogenic Activity in Guinea Pigs

A dose-related decrease of supracaudal gland area was demonstrated in the guinea pig after topical application of 25, 100 or 400 μg Win 17665 for 3 weeks. During this time dosing and measurement were done without knowledge of the treatment applied. Figure 2 shows, however, that at 3 weeks the inhibitory effect of Win 17665 at 400 μg/gland was no greater than that observed with 100 μg/gland.

Seminal vesicle and adrenal gland weights were unaffected after 3 weeks of topical administration of Win 17665, suggesting that, under these conditions, Win 17665 did not produce systemic effects on these organs.

DISCUSSION AND CONCLUSIONS

Win 17665, applied topically, produced a dose-related reduction in size, weight, cholesterol content and degree of development of the flank organ of the sexually intact male hamster. When topically applied to the flank organ on one side, the flank organ on the other side was unaffected. Reduction in the above measurements was reflected by a decrease in the area and lipid droplet content of histologic sections from treated flank organs. Topically applied Win 17665 was also an antagonist of TP, DHT, and AED each topically administered to the hamster flank organ. This indicates potential antagonism of a variety of androgens formed by the testes, adrenals or ovaries and antagonism of the form of testosterone that stimulates peripherally sensitive target sites.

Subcutaneous administration of Win 17665 at 100 mg/kg, but not at 10 mg/kg, revealed that this compound reduced the size of the seminal vesicles indicating general antiandrogenic properties. It was interesting that systemic antiandrogenic activity did not occur upon oral treatment or on massive topical doses of Win 17665. Since this antiandrogen had to penetrate the skin

TABLE V. *Effect of Win 17665 on flank organs of castrated hamsters stimulated by testosterone propionate (TP), 5α-dihydrotestosterone (DHT) or 4-androstene-3,17-dione (AED)*

Topical treatment flank organ/day × 14[a]	Flank organ parameters ± SE			
	Mean diameter (mm)	Mean weight (mg)	Mean cholesterol (μg)	Median development score[b]
Vehicle	2.68 ± 0.13	6.85 ± 0.19	22.87 ± 1.09	0
1 μg TP (as base)	4.80 ± 0.16	33.79 ± 2.39	162.7 ± 6.67	4
1 μg TP + 25 μg Win 17665	4.14 ± 0.26[c]	21.83 ± 1.69[d]	108.4 ± 4.36[e]	3[c]
1 μg TP + 75 μg Win 17665	4.14 ± 0.28[c]	21.70 ± 1.99[d]	106.2 ± 9.24[e]	3[d]
1 μg TP + 225 μg Win 17665	3.76 ± 0.19[c]	15.02 ± 1.24[e]	77.37 ± 5.32[e]	2[e]
Vehicle	2.83 ± 0.10	4.25 ± 0.45	17.00 ± 1.16	0
1 μg DHT	4.59 ± 0.37	29.27 ± 4.14	114.6 ± 4.59	4
1 μg DHT + 25 μg Win 17665	3.98 ± 0.22	21.42 ± 2.43	96.50 ± 4.1[c]	3
1 μg DHT + 75 μg Win 17665	4.22 ± 0.09	16.32 ± 1.74[c]	87.62 ± 6.26[d]	2
1 μg DHT + 225 μg Win 17665	3.61 ± 0.17[c]	5.49 ± 0.73[e]	40.62 ± 3.71[e]	1.5[c]
Vehicle	2.73 ± 0.11	6.06 ± 0.38	22.50 ± 1.71	0.75
5 μg AED	4.43 ± 0.20	21.82 ± 1.60	115.1 ± 7.04	3.5
5 μg AED + 25 μg Win 17665	3.59 ± 0.25[d]	9.28 ± 0.75[e]	66.62 ± 4.69[e]	2.0[c]
5 μg AED + 75 μg Win 17665	3.47 ± 0.9[e]	7.39 ± 0.58[e]	59.75 ± 5.17[e]	2.0[d]
5 μg AED + 225 μg Win 17665	3.10 ± 0.19[e]	5.72 ± 0.39[e]	49.00 ± 5.61[e]	1.25[c]

[a] Each group had 8 animals. Both flank organs received the same treatment. Hamsters were treated twice on Mon. and Fri., once on Tues. through Thurs. and not on Sat. or Sun. The experiments employing TP and AED, but not DHT, as androgens were done blind.

[b] Scored between 0 for barely visible to a high of 4 for maximum development.

[c] $P < 0.05$: [d] $P < 0.01$; [e] $P < 0.001$ compared with the mean for group treated with respective androgen.

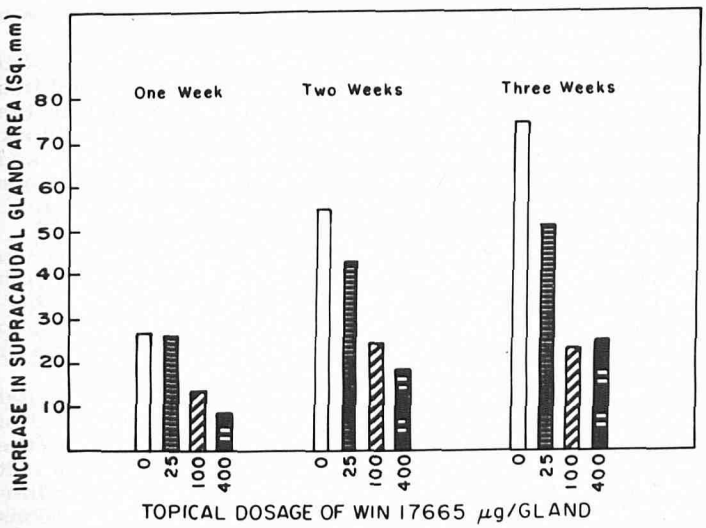

FIG 2. Effect of repeated topical Win 17665 treatment on the area of the supracaudal gland of guinea pigs.

to the dermal layer in order to exert its activity, it is quite possible that Win 17665 was metabolized to a form that is inactive systemically. The most logical metabolite, the 5α-dihydro derivative of Win 17665, was found to be inactive in our animal model. Therefore, steroid 5α-reductase may be the enzyme responsible for preventing systemic antiandrogenic activity on topical application.

Besides reporting the antiandrogenic activity of Win 17665 on the hamster flank organ and guinea pig supracaudal gland *per se*, we also described a reliable means of appraising the action of topically applied androgens and antiandrogens in those models. Simultaneous examination of internal endocrine organs after a course of topical treatment gives a direct estimate of systemic hormonal activity after percutaneous absorption. In experiments beyond the scope of this paper, we have noted that glucocorticoids applied topically reduced the weight and development score but not the diameter or cholesterol content of the flank organ, enabling a distinction to be made between antiandrogens and glucocorticoids.

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